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Novel cyclized Pifithrin-α p53 inactivators: synthesis and biological studies

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Abstract—Starting from various cyclic or bicyclic ketones, we have synthesized novel Pifithrin-α analogues bearing different methyl substituted phenyl ketone groups at the N_3-position of the 2-iminothiazole heterocycle. From stability studies in a biological medium as well as under specific chemical conditions, we have shown by NMR techniques that through a dehydration process, some derivatives can generate their corresponding cyclized analogues. All of the new analogues, Pifithrin-like and polycyclic dehydrated derivatives were assessed for their p53 inactivation potency by measuring survival of cortical neurons, whose death was induced by the DNA-damaging agent etoposide. Pifithrin-α like **2f** as well as the cyclic dehydrated **6b** analogue were found to be one log more potent p53 inactivators than reference compound Pft-α, with EC_{50} values ranging around 30 nM. These results support the finding that p53 inactivation by Pft-α analogues could be also due to the presence of the cyclic dehydrated Pft-α forms, generated in situ in the biological assay incubation medium.

1. Introduction

During the last few years, p53 inactivators have received increasing attention due to their possible application in several major pathologies such as neurodegenerative disorders (Alzheimer's disease, Parkinson's disease and stroke),[1] cancer therapy[2] and other pathologies related to various signaling pathways.[3] Pft-α **1** (Fig. 1) is the leading compound of the known p53 inactivators. It was isolated by screening of a chemical library in a cell-based read-out system for its ability to reduce p53-dependent transactivation.[2]

In a recent publication,[1] 14 new Pft-α analogues bearing various methylene substituted aromatic ketones at the N_3-position of the Pft-α scaffold have been described. These analogues were found to be highly potent in protecting PC12 cells or primary hippocampal neurons against DNA-damaging agent induced death. These new derivatives were primarily synthesized to assess the importance of the electrodonating and electrowithdrawing substitution of the aromatic moiety of the substituent at the N_3-position of the bicyclic backbone. In this current study, we were interested in the design of new Pft-α like derivatives in which, on the one hand, the tetrahydrobenzo moiety was replaced by its octahydronaphtho counterpart and, on the other hand, new substituents were introduced at the N_3-position of the heterocycle, such as a cyano group on the methylene group on the acetophenone moiety (Fig. 1).

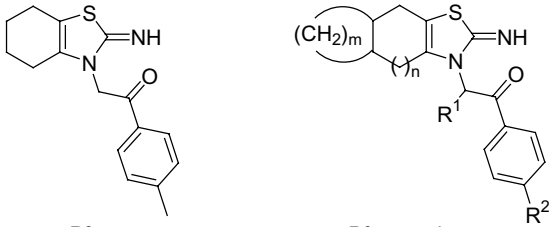

Figure 1. General structure of new p53 inactivators.

Keywords: Pft-α; Cortical neuron survival; p53 Inactivators; Dehydration reaction.
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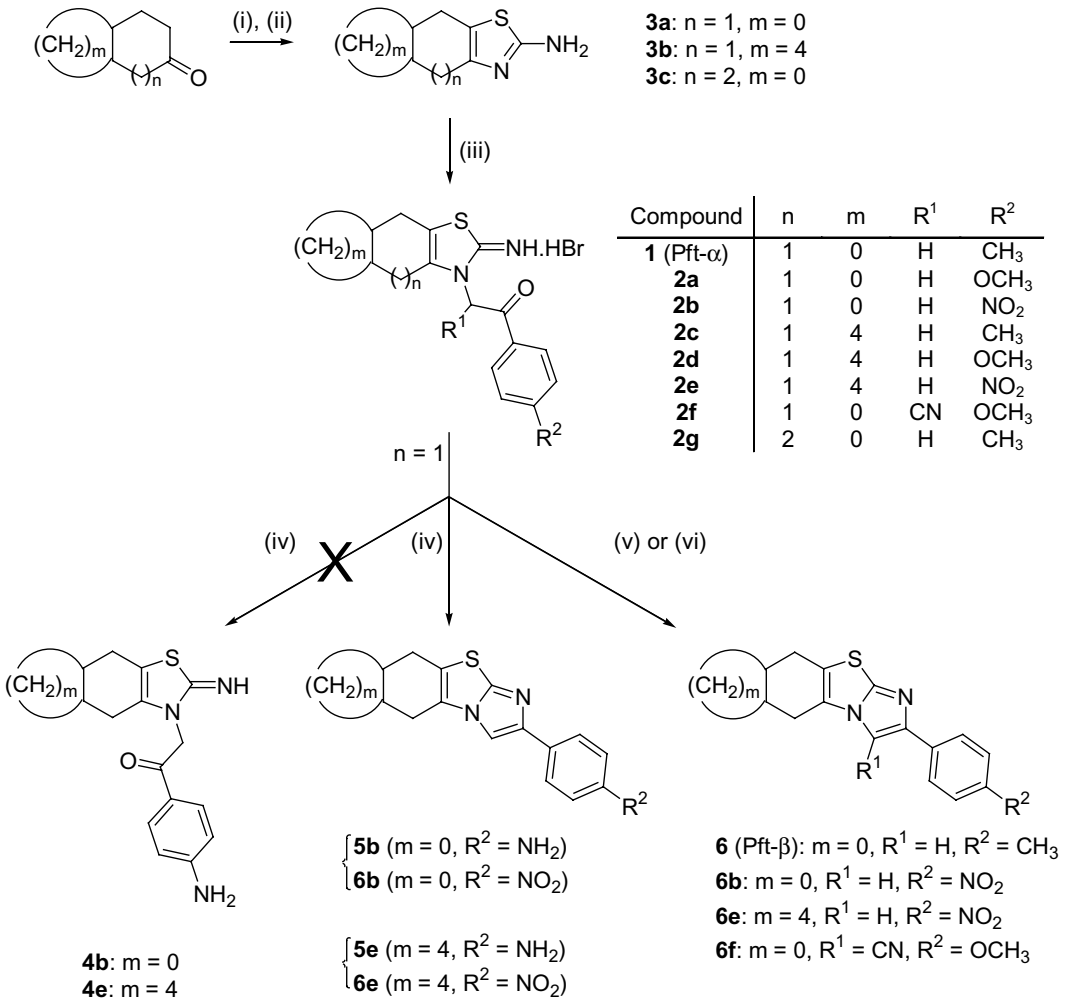
In the course of our synthetic efforts to reach those Pft-α derivatives, we discovered that some of the desired compounds were dehydrated into their corresponding cyclic imidazothiazole derivatives. We report the synthesis of new Pft-α analogues, their chemical and biological stabilities, as well as their p53 inhibitory activities.

2. Synthesis and chemical stability

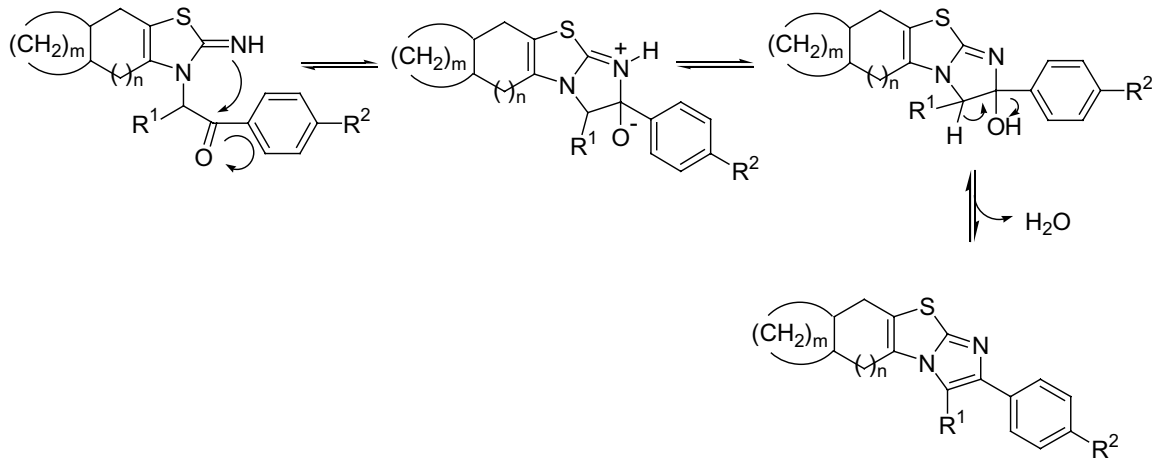
The synthesis of 2-aminothiazole hydroiodide salt precursors was carried out according to the method described by King and Hlavacek.[4] The solvent free reaction was performed by heating various cyclic ketones (cyclohexanone, 2-decalone, cycloheptanone) with thiourea in the presence of iodine. Basic treatment of the hydroiodide salt allowed the isolation of the corresponding free aminothiazoles **3a**–**c**, which could be N-alkylated on the endocyclic N_3-position by the selected α-bromoacetophenone at room temperature in toluene. The resulting N_3-substituted 2-iminothiazole derivatives **1**, **2a**–**g** were isolated in yields ranging from 40% to 70% after purification by crystallization. Their respective structures were assigned through NMR and MS analysis (Scheme 1).[5]

Subsequently, the nitro Pft-α derivatives **2b** and **2e** were submitted to reduction in order to isolate their aniline analogues. This reduction process involved the use of Fe/NH_4Cl as the reductant in refluxed protic solvents.[6] We discovered that instead of the expected amino derivatives **4b** and **4e**, we isolated mostly their dehydrated tricyclic amino derivatives, **5b** and **5e**, and their corresponding tricyclic nitro analogues **6b** and **6e**.

These unexpected results were confirmed by stability study of the opened form of the nitro Pft-α analogues **2b** and **2e** in a protic solvent (EtOH, *i*-PrOH). The corresponding tricyclic nitro derivatives **6b** and **6e** resulting from a dehydration process as suggested in Scheme 2 were isolated in quantitative yields and fully characterized. The ability of Pft-α analogues to undergo this dehydration process depends on the electrodonating or electrowithdrawing properties of the phenyl ring



Scheme 1. Reagents and conditions: (i) thiourea, I_2, 110 °C, 12 h; (ii) saturated Na_2CO_3, H_2O; **3a** (52%), **3b** (47%), **3c** (37%), for two steps; (iii) 4-R^2-C_6H_4-C(O)-CH(R^1)Br, toluene, rt, 48 h; **1** (54%), **2a** (61%), **2b** (55%), **2c** (80%), **2d** (41%), **2e** (47%), **2f** (55%), **2g** (40%); (iv) NH_4Cl, Fe, EtOH/H_2O (5/3, v/v), reflux, 2 h; mixture of **5b** and **6b** (9/1); mixture of **5e** and **6e** (9/1); (v) MeOH, rt; quantitative for **6b** and **6e**; (vi) biological medium, 37 °C; quantitative for **6**, **6b**, **6e**, **6f**.



Scheme 2.

substituents. Compounds with withdrawing substituents such as a nitro group on the phenyl ring, **2b** and **2e**, were found more sensitive to dehydration (few hours at room temperature) than analogues bearing electrodonating substituents, which needed a longer time to cyclize (one night in refluxed MeOH). These new cyclic 2-imidazothiazoles represent new substrates suitable for further new N-acylation or N-alkylation reactions.

The ability of Pft-α analogues to be dehydrated into their corresponding cyclic forms led us to study their biological stabilities.

3. Biological stability

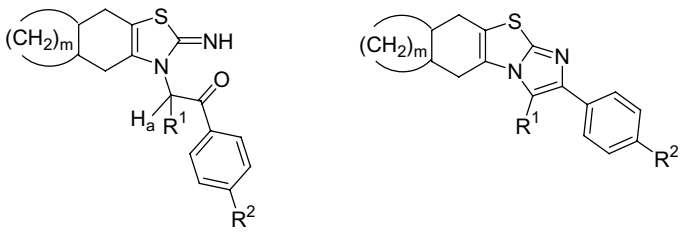
The ability of the new analogues to be dehydrated in biological experimental conditions leading to the corresponding cyclized analogues, questions about the possibility for these Pft-α cyclic forms to be the bioactive intermediates for p53 inactivation. We selected three compounds, nitro derivatives **2b** and **2e** and the α-cyano compound **2f** as well as the reference compound Pft-α **1** to be incubated for several hours at 37 °C in the biological medium used in the p53 inhibition assay (DMEM serum, horse serum and glutamate).[1] After extraction from the biological medium, the residues were analyzed by NMR.

The NMR analysis showed that the signal corresponding to the protons located at the α-position of the carbonyl function ($\delta = 4.7$ ppm) disappeared. At the same time, a single proton signal corresponding to the newly formed fused cyclic imidazole appeared at 7.3 ppm. In the case of the α-cyano derivative, we noticed the complete absence of the signal corresponding to the open form.

The ^{1}H NMR characteristic chemical shifts of the acyclic and cyclic Pft-α derivatives are summarized in Table 1.

The stability of the opened form in the assay could be characterized by the values of the corresponding half-reaction times. These values, reported in Table 2 varied noticeably for the different compounds tested: **1**, **2b**, **2e** and **2f**. Analogue **2f** bearing a cyano group at the α-position of the keto function and a methoxy group on the

Table 1. ^{1}H NMR characteristic shifts of Pft open and cyclic forms



open form cyclic dehydrated form

Open form → cyclic form	*m*	R^1	R^2	$\delta(H_a)$ ppm	$\delta(R^1)$ ppm
1 → 6	0	H	CH_3	4.7	7.3
2b → 6b	0	H	NO_2	4.8	7.3
2e → 6e	4	H	NO_2	4.8	7.4
2f → 6f	0	CN	OCH_3	5.7	—

Table 2. Stabilities of new Pifithrin-like derivatives in biological medium[a]

Compound	*m*	R^1	R^2	$t_{1/2}$ (h)
1	0	H	CH_3	4 ± 0.5
2b	0	H	NO_2	6 ± 0.5
2e	4	H	NO_2	5 ± 0.5
2f	0	CN	OCH_3	12 ± 0.5

[a] DMEM, horse serum, glutamate.

phenyl ring, appears to be the most stable analogue ($t_{1/2}$ = 12 h) while **1** and analogues **2b** and **2e** have $t_{1/2}$ values less than 6 h.

4. Biological activity: p53 inactivation

The newly synthesized compounds from both series, Pft-α analogues as well as cyclic dehydrated analogues, were assayed for their ability to protect mouse embryo cortical neurons against a DNA-damaging agent.[1] Pft-α was used as external control (EC_{50} = 300 nM). In response to etoposide used as death inducer, neuronal survival, expressed in EC_{50} value, was determined for all the tested compounds. Compounds **2f** and **6b** were found to be the most active analogues, with EC_{50} values around 30 nM.

In conclusion, as far as the two most potent p53 inactivators are a Pft-α analogue (**2f**) and a cyclic dehydrated analogue (**6b**), it could be suggested that p53 inactivation by Pft-α analogues could be due in part to the cyclic dehydrated forms, which are generated in situ during the incubation time in the biological medium. Additional experiments are underway in order to verify this hypothesis.

Acknowledgements

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5. Open form analogues: **1** (Pft-α): ^{1}H NMR (250 MHz, DMSO-d_6) δ 9.60 (s, 1H), 7.95 (d, 2H, *J* = 8.2 Hz), 7.45 (d, 2H, *J* = 8.2 Hz), 4.70 (s, 2H), 2.51–2.31 (m, 5H), 1.83–1.79 (m, 2H), 1.36 (br s, 1H), 1.02 (d, 3H, *J* = 6.5 Hz). Anal. (Found: C, 52.33; H, 5.48; N, 7.91. $C_{16}H_{18}N_2OS$·HBr requires C, 52.32; H, 5.21; N, 7.63). ES/MS *m*/*z* 287 $(M+H)^+$. **2a**: ^{1}H NMR (250 MHz, DMSO-d_6) δ 9.47 (s, 1H), 8.03 (d, 2H, *J* = 8.9 Hz), 7.16 (d, 2H, *J* = 8.9 Hz), 4.67 (s, 2H), 3.89 (s, 3H), 2.55 (m, 2H), 2.32 (m, 2H), 1.73 (m, 4H). Anal. (Found: C, 50.26; H, 4.83; N, 7.35. $C_{16}H_{18}N_2O_2S$·HBr requires C, 50.14; H, 5.00; N, 7.31). ES/MS *m*/*z* 303 $(M+H)^+$. **2b**: ^{1}H NMR (250 MHz, DMSO-d_6) δ 9.54 (s, 1H), 8.46 (d, 2H, *J* = 8.9 Hz), 8.28 (d, 2H, *J* = 8.9 Hz), 4.80 (s, 2H), 2.56–2.37 (m, 4H), 1.74 (m, 4H). Anal. (Found: C, 45.52; H, 3.82; N, 10.52. $C_{15}H_{15}N_3O_3S$·HBr requires C, 45.24; H, 4.05; N, 10.55). ES/MS *m*/*z* 318 $(M+H)^+$. **2c**: ^{1}H NMR (250 MHz, DMSO-d_6) δ 9.71 (s, 1H), 8.34 (d, 2H, *J* = 8.9 Hz), 8.18 (d, 2H, *J* = 8.9 Hz), 4.80 (s, 2H), 2.61–1.20 (m, 17H). Anal. (Found: C, 57.17; H, 6.18; N, 6.42. $C_{20}H_{24}N_2OS$·HBr requires C, 57.00; H, 5.98; N, 6.65). ES/MS *m*/*z* 341 $(M+H)^+$. **2d**: ^{1}H NMR (250 MHz, DMSO-d_6) 9.47 (s, 1H), 8.03 (d, 2H, *J* = 8.9 Hz), 7.16 (d, 2H, *J* = 8.9 Hz), 4.67 (s, 2H), 3.89 (s, 3H), 2.55–1.25 (m, 14H). Anal. (Found: C, 55.15; H, 5.46; N, 6.35. $C_{20}H_{24}N_2O_2S$·HBr requires C, 54.92; H, 5.76; N, 6.40). ES/MS *m*/*z* 357 $(M+H)^+$. **2e**: ^{1}H NMR (250 MHz, DMSO-d_6) δ 9.71 (s, 1H), 8.34 (d, 2H, *J* = 8.9 Hz), 8.18 (d, 2H, *J* = 8.9 Hz), 4.80 (s, 2H), 2.61–1.20 (m, 14H). Anal. (Found: C, 50.52; H, 4.65; N, 9.19. $C_{19}H_{21}N_3O_3S$·HBr requires C, 50.45; H, 4.90; N, 9.29). ES/MS *m*/*z* 372 $(M+H)^+$. **2f**: ^{1}H NMR (250 MHz, $CDCl_3$) δ 9.57 (s, 1H), 7.91–8.05 (m, 2H), 6.88–7.00 (m, 2H), 5.65 (s, 1H), 3.84 (s, 3H), 2.55 (m, 2H), 2.32 (m, 2H), 1.73 (m, 4H). Anal. (Found: C, 49.85; H, 4.46; N, 10.07. $C_{17}H_{17}N_3O_2S$·HBr requires C, 50.01; H, 4.44; N, 10.29). ES/MS *m*/*z* 328 $(M+H)^+$. **2g**: ^{1}H NMR (250 MHz, DMSO-d_6) δ 9.60 (s, 1H), 7.95 (d, 2H, *J* = 8.2 Hz), 7.45 (d, 2H, *J* = 8.2 Hz), 4.22 (s, 2H), 2.65–2.50 (m, 7H), 1.74 (m, 6H). Anal. (Found: C, 53.71; H, 5.32; N, 7.52. $C_{17}H_{20}N_2OS$·HBr requires C, 53.54; H, 5.55; N, 7.35). ES/MS *m*/*z* 301 $(M+H)^+$.
Cyclized form analogues: **6** (Pft-β): ^{1}H NMR (250 MHz, DMSO-d_6) δ 7.95 (d, 2H, *J* = 8.2 Hz), 7.45 (d, 2H, *J* = 8.2 Hz), 7.32 (s, 1H), 2.51–2.31 (m, 5H), 1.83–1.79 (m, 2H), 1.36 (br s, 1H), 1.02 (d, 3H, *J* = 6.5 Hz). Anal. (Found: C, 71.75; H, 5.82; N, 10.45. $C_{16}H_{16}N_2S$ requires C, 71.60; H, 6.01; N, 10.44). ES/MS *m*/*z* 269 $(M+H)^+$. **6b**: ^{1}H NMR (250 MHz, DMSO-d_6) δ 7.65 (d, 2H, *J* = 8.5 Hz), 7.45 (s, 1H), 7.15 (d, 2H, *J* = 8.5 Hz), 2.40–2.50 (m, 4H), 1.80–1.95 (m, 4H). Anal. (Found: C, 60.38; H, 4.46; N, 14.28. $C_{15}H_{13}N_3O_2S$ requires C, 60.18; H, 4.38; N, 14.04). ES/MS *m*/*z* 300 $(M+H)^+$. **6e**: ^{1}H NMR (250 MHz, DMSO-d_6) δ 7.65 (d, 2H, *J* = 8.5 Hz), 7.45 (s, 1H), 7.15 (d, 2H, *J* = 8.5 Hz), 2.55–1.25 (m, 14H). Anal. (Found: C, 64.64; H, 5.32; N, 11.95. $C_{19}H_{19}N_3O_2S$ requires C, 64.57; H, 5.42; N, 11.89). ES/MS *m*/*z* 354 $(M+H)^+$. **6f**: ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.91–8.05 (m, 2H), 6.88–7.00 (m, 2H), 3.84 (s, 3H), 2.55 (m, 2H), 2.32 (m, 2H), 1.73 (m, 4H). Anal. (Found: C, 66.14; H, 5.12; N, 13.45. $C_{17}H_{15}N_3OS$ requires C, 66.00; H, 4.89; N, 13.58). ES/MS *m*/*z* 310 $(M+H)^+$.
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